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Low Aerobic Capacity and High-Fat Diet Contribute to Oxidative Stress and IRS-1 Degradation in the Kidney

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Key Words
IRS-1 · Reactive oxygen species · Aerobic capacity · Insulin resistance

Abstract
Background/Aims: Insulin receptor (IR-α and IR-β) is reduced in the kidney of insulin-resistant rodents. It is unknown if there are also reductions in insulin receptor substrate (IRS)-1 or if these effects are due to metabolic injury. Thereby, we hypothesized intrinsically high aerobic fitness would protect against high-fat diet (HFD)-induced reactive oxygen species (ROS) and IRS-1 degradation. ***Methods:*** We investigated the effects of HFD on triglyceride content, ROS production and IRS-1 degradation in the kidney of high-capacity (HCR)/low-capacity (LCR) rats, a model of intrinsic high and low aerobic capacity. Eighteen-week-old HCR and LCR rats were placed on a HFD or normal chow diet for 7 weeks. Intraperitoneal glucose tolerance, ROS, IR-β, total IRS-1 and ubiquitination were measured. ***Results:*** The HCR displayed greater insulin sensitivity and were resistant to HFD-induced insulin resistance. In the LCR kidney, HFD increased ROS potential, and reduced total IR-β and IRS-1 without altering triacylglycerol content. IRS-1 ubiquitination was higher in the LCR than HCR kidney, increased after HFD. ***Conclusions:*** Our data support that HFD-mediated kidney ROS is associated with reductions in IRS-1 and systemic insulin resistance. Further, high intrinsic aerobic capacity protects against IRS-1 degradation in the kidney following exposure to HFD.

Introduction

A large body of evidence supports the adverse effects of a high-fat diet (HFD) on systemic insulin sensitivity/glucose homeostasis and insulin action in traditional (for example skeletal muscle) as well as nontraditional (for example cardiovascular) insulin sensitive tissues in both humans and animal studies. Recent data support a role for insulin resistance and glucose intolerance in chronic kidney disease (CKD) [1–5]. Indeed, recent data suggest that the kidney is an insulin-sensitive tissue and that insulin metabolic signaling is important for maintaining

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normal renal structural and functional integrity. However, to date, the possible mechanisms that alter kidney insulin metabolic signaling, as it contributes to insulin resistance, have not been fully elucidated.

Considerable evidence indicates that exercise and aerobic capacity may maintain insulin metabolic sensitivity even in obese and insulin-resistant persons and, recently, in those with CKD [6–8]. Indeed, reports of the National Health and Nutrition Examination Survey (NHANES) III suggest increased physical activity contributes to normal glomerular filtration [9]. Furthermore, decreased physical fitness has been documented as an important mediator of insulin resistance in humans [10] and animals [11]. Importantly, the association between decreased aerobic capacity and diminished insulin metabolic sensitivity has been demonstrated in those with early CKD [12].

Insulin resistance has been ameliorated in rodent models following reductions in oxidative stress in insulin-sensitive tissues [13] and exacerbated by an increase in oxidative stress due to a HFD [14]. Increased reactive oxygen species (ROS) formation has been implicated in many renal structural and functional abnormalities [15] and is believed to be a primary contributor to diabetic kidney disease [16]. With multiple potential cellular sources of ROS production and multiple potential effectors, the elucidation of individual effects on the kidney observed during increased oxidative stress is complex. Current evidence suggest increased oxidative stress leads to alterations in insulin signaling through serine phosphorylation of the insulin receptor substrate-1 (IRS-1) docking protein in various insulin-sensitive tissues [17]. One hypothesis for decreased insulin signaling in states of high oxidative stress could be decreased protein content of IRS-1 due to increased IRS-1 ubiquitin proteosome-dependent degradation as is observed with increased serine phosphorylation of IRS-1 [18, 19]. Thereby, we examined the oxidative stress potential and content of insulin signaling proteins in kidney tissue of animals possessing differing phenotypes of aerobic capacity, and whether exposure to a metabolic challenge (that is, HFD) would contribute to IRS-1 degradation.

Methods

Animal Protocol

All animal procedures were approved by the University of Missouri animal care and use committees and housed in accordance with National Institutes of Health (NIH) guidelines. The creation of the high-/low-capacity rat (HCR/LCR) model of high and low intrinsic aerobic capacity has been described previously [20] and several phenotypic characteristics of the divergent phenotypes have been investigated [11, 21]. Briefly, bidirectionally selected lines were generated from a founder population of 80 male and 88 female N:NIH stock rats based on intrinsic aerobic treadmill running capacity. Thirteen families for each line were set up for a within-family rotational breeding paradigm that keeps the inbreeding at <1% per generation. At each generation, young adult rats (11 weeks of age) were tested for their inherent ability to perform forced speed-ramped treadmill running until exhausted. This test was performed daily over 5 consecutive days. The greatest distance in meters achieved out of the 5 trials was considered the best estimate of an individual's aerobic exercise capacity [20]. The highest scored female and male from each of the 13 families were selected as breeders for the next generation of high-capacity runners (HCR). The same process was used with lowest scored females and males to generate low-capacity runners (LCR). Female HCR and LCR rats (generation 20, 18 weeks of age) used for this investigation were housed in pairs in a temperature-controlled environment with a 12-hour light/dark cycle. The exercise performance test results for these animals are displayed in table 1. The 2 phenotypes were distributed into 2 groups based on equal distribution of run time to exhaustion and running distance and fed ad lib either a chow (17% energy from fat; Formulab Diet 5008) or HFD (60% energy from fat; TestDiet 58G9) for 7 weeks.

Intraperitoneal Glucose Tolerance Test

Following an overnight fast, animals were phlebotomized from the tail vein to determine basal fasted plasma glucose and insulin levels. A glucose challenge (2 g/kg) was administered via intraperitoneal injection, and additional blood samples were collected at 15, 30, 45, 60 and 120 min. All blood was collected into tubes containing potassium EDTA as an anticoagulant, stored on ice and centrifuged at 4°C to prepare plasma samples that were stored at –80°C. Glucose levels were determined on plasma samples using Trace/DMA Glucose Oxidase (Thermo, Waltham, Mass., USA) and insulin levels were determined using a rat insulin ELISA kit (Linco Research, St. Charles, Mo., USA).

Serum Analysis

Serum levels of nonesterified free fatty acids (Wako Chemicals, Richmond, Va., USA) and triacylglycerols (Sigma, St. Louis, Mo., USA) were determined on serum prepared from blood collected during terminal exsanguinations which were allowed to clot on ice and centrifuged at 4°C before being stored at –80°C.

Tissue Homogenization Procedure

Kidney tissue was removed from anesthetized rats and placed in ice-cold sucrose homogenization buffer (SHB; 0.25 M sucrose, 0.5 mM EDTA, 50 mM HEPES, pH approx. 7.5) containing protease (Complete; Roche Diagnostics, Mannheim, Germany) and phosphatase inhibitors (Cocktail-1 and Cocktail-2; Sigma). The tissue was homogenized and cellular fractionation was performed as previously described [22–24]. Briefly, approximately 400–500 mg of tissue was homogenized in a Dounce glass-on-glass homogenizer. The crude homogenate was centrifuged (1,500 *g* for 10 min) at 4°C with the supernatant reserved as the whole homogenate. A portion of the whole homogenate underwent ultracentrifugation (33,000 *g* for 30 min, 4°C) and the supernatant was transferred to another tube for further ultracentrifugation (100,000 *g* for 1 h, 4°C). The 33,000 *g* pellet was resuspended in SHB and la-

beled as the crude mitochondrial pellet. The 100,000 *g* supernatant was labeled as cytosolic fraction, and the pellet was resuspended in SHB and labeled as the plasma membrane fraction. Protein concentration for all homogenates and fractions were determined by a modified Bradford assay (Coomasie Plus; Pierce, Rockford, Ill., USA) and stored at –80°C.

Fat Pad Collection

Omental and periovarian adipose tissue pads were removed and individually weighed. The body composition ratio described is the cumulative mass of the fat pads divided by the weight of each animal: (omental + periovarian)/body mass.

Kidney Lipid Content

The lipid content of kidney tissue was determined as previously described [25]. Powdered kidney (30 mg) was added to 1 ml of lipid extraction solution composed of 1:2 vol/vol methanol-chloroform, homogenized for 30 s, and exposed to gentle agitation overnight at 4°C. One milliliter of 4 mM MgCl was added, vortexed and centrifuged for 1 h at 1,000 *g* at 4°C. The organic phase was removed, evaporated, and reconstituted in butanol-Triton X-114 (3:2 vol/vol) and vortexed. Lipid content was measured from a commercially available kit (F6428; Sigma), and TG concentration was expressed as nanomoles per gram wet weight.

Immunoprecipitation and Western Blotting

For immunoprecipitation, 100 μg of whole homogenate was incubated for 1 h with 2 μl of primary antibody ubiquitin (Santa Cruz Biotechnologies, Santa Cruz, Calif., USA) in lysis buffer (1% Triton X-100, 100 mM NaCl, 20 mM Tris, 2 mM EDTA, 10 mM $MgCl_2$, 10 mM NaF, protease inhibitors, approx. pH 7.5) at 4°C. Thirty milliliters of agarose beads (Santa Cruz Biotechnologies) were added and the samples incubated overnight. The beads were pelleted and washed 5 times with ice-cold PBS (1% NP-40, protease inhibitors). Samples were boiled in loading buffer and analyzed as described below. For Western blotting, fractions and whole homogenates were analyzed under denaturing conditions with SDS-PAGE using a Criterion electrophoresis system (Bio-Rad, Hercules, Calif., USA). Protein (30 μg) was mixed with 5 μl of loading buffer and incubated at 100°C for 5 min. Samples were then loaded in wells of 5% (IRS-1, IR-β) or 15% (cytochrome c) precast gels (Bio-Rad) and run at 180 V for 45–55 min. Proteins were transferred onto a PVDF membrane in a wet transfer system (Bio-Rad) at 100 V for 1 h. Blots were blocked with 5% nonfat dry milk in TBST overnight. Membranes were incubated with primary antibodies IRS-1 (rabbit polyclonal) at 1:500 (Santa Cruz Biotechnologies), anti-IR-β (rabbit polyclonal) at 1:500 (Santa Cruz Biotechnologies) and anti-cytochrome c (mouse monoclonal) at 1:500 (Upstate Biotechnology, Lake Placid, N.Y., USA) in 3% nonfat dry milk in TBST overnight. Membranes were washed and incubated for 60 min with secondary antibody (1:5,000) in 3% nonfat dry milk in TBST, using anti-rabbit horseradish peroxidase (HRP)-linked antibody (H&L; Cell Signaling, Danvers, Mass., USA) for IRS-1 and IR-β, and HRP-conjugated goat anti-mouse antibody (H&L; Jackson Immuno Research Laboratories, Westgrove, Pa., USA) for cytochrome c. Blots were incubated with enhanced chemiluminescence for 1 min, and the protein bands were visualized by use of ChemiDoc XRS (Bio-Rad) and quantified by Quantity One 1-D analysis software (Bio-Rad). Equal loading of total proteins in each lane was checked by Amido black staining using a densitometer.

ROS Potential

The formation of ROS was evaluated in kidney tissue by lucigenin-enhanced chemiluminescence, as previously described [23]. Kidney tissue whole homogenate (100 μl) was added to 1.4 ml of 50 mM phosphate (KH_2PO_4) buffer (150 mM sucrose, 1 mM EGTA, 5 μM lucigenin, 100 μM NADPH, pH 7.0) in dark-adapted counting vials. After dark adaptation for 1 h, the samples were counted every 20 s for 3 min using a luminometer (Autolumat Plus LB953; Berthold Technologies, Bad Wildbad, Germany). The samples were then normalized to total protein in the whole homogenate. Values are expressed as relative light units per minute per milligram protein.

NAD(P)H Oxidase Activity

NAD(P)H oxidase activity was determined in plasma membrane fractions as previously described [26]. Aliquots of plasma membrane fraction were incubated with NAD(P)H (100 μM) at 37°C. NAD(P)H activity was determined by measuring the conversion of Radical Detector (Cayman Chemical, Ann Arbor, Mich., USA) using spectrophotometric (450 nm) techniques.

Citrate Synthase Activity

Citrate synthase activity was determined as previously described in detail [27]. Briefly, kidney mitochondrial fractions were freeze-thawed 3 times and were incubated in the presence of oxaloacetate, acetyl-CoA, and DTNB. Spectrophotometric detection of reduced DTNB at a wavelength of 412 nm served as an index of enzyme activity.

Cytochrome c Oxidase Activity

Cytochrome c oxidase activity was measured colorimetrically by a commercially available kit (Sigma). The assay measures the oxidation of ferrocytochrome c to ferricytochrome c by cytochrome c oxidase, and enzyme activity is expressed as units per milliliter per gram protein.

Protein Carbonylation

Measurement of reactive aldehydes was performed as previously described [28]. Briefly, kidney tissue samples were homogenized in coupling buffer (100 mM sodium acetate, 20 mM NaCl, 0.1 mM EDTA, pH 5.5) supplemented with protease inhibitors (Complete; Roche Diagnostics). The homogenate was ultracentrifuged (100,000 *g* for 1 h) at 4°C, and the supernatant was collected. The protein concentration of the resulting supernatant was determined by modified Bradford assay (Coomasie Plus; Pierce). Fifty micrograms of sample was incubated with a final concentration of 0.5 mM EZ-link biotin hydrazide (5 mM stock prepared fresh in DMSO; Pierce) in coupling buffer for 2 h at room temperature. SDS-PAGE of the samples using 15% polyacrylamide was performed, and the proteins were transferred to PVDF membrane. The membranes were blocked overnight at 4°C in 5% nonfat dry milk in TBST overnight, and incubated for 1 h at room temperature with HRP-conjugated streptavidin (1:5,000 dilution). The biotin-avidin interaction was detected by electrochemiluminescence. The protein bands were visualized by use of ChemiDoc XRS (Bio-Rad) and quantified by Quanity One 1-D analysis software (Bio-Rad).

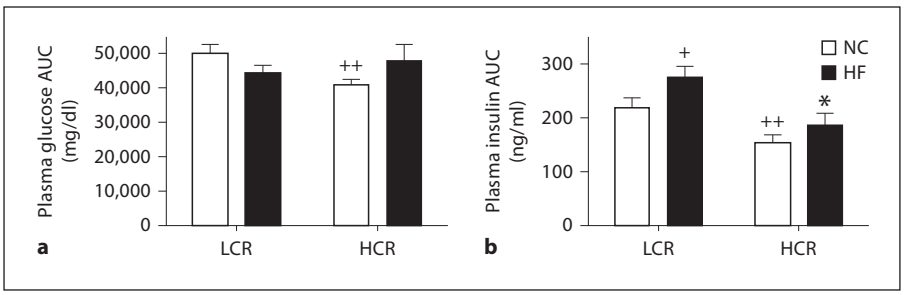

Fig. 1. Calculated AUC during intraperitoneal glucose tolerance test for glucose (**a**) and insulin (**b**). $^{+}$ $p < 0.05$ between NCD and HFD; $^{++}$ $p < 0.05$ between LCR and HCR on NCD; * $p < 0.05$ between LCR and HCR on HFD.

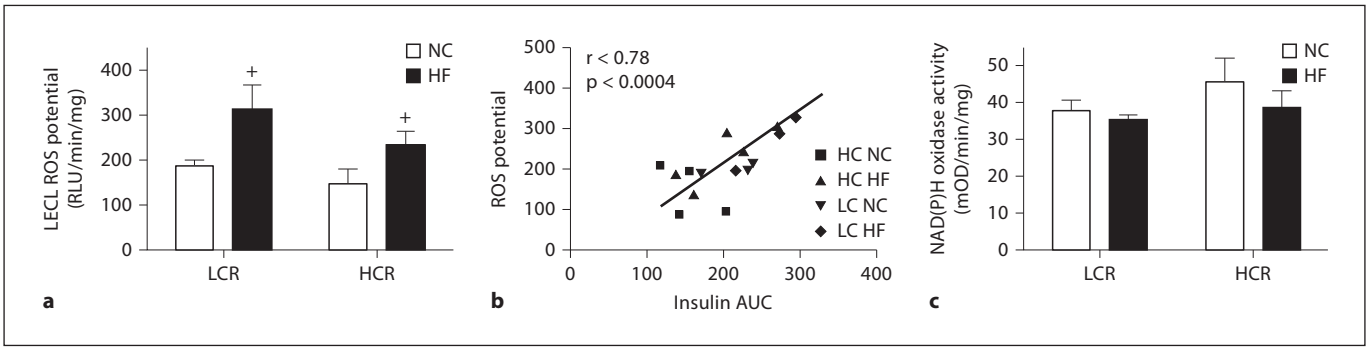

Fig. 2. Kidney ROS potential. **a** Lucigenin-enhanced chemiluminescence (LECL) ROS potential. **b** Correlation of ROS potential and insulin AUC across all groups. **c** NAD(P)H oxidase activity from isolated plasma membrane fractions of fresh kidney tissue homogenates. $^{+}$ $p < 0.05$ between NCD and HFD.

Statistics

All values are expressed as means ± SE. Statistical analyses were performed using SPSS version 13.0 (SPSS Inc., Chicago, Ill., USA). ANOVA was followed by Fisher's least significant difference test for multiple comparisons and Student's t test was used for paired analysis.

Results

Aerobic Capacity, Total Body Weight, Serum and Kidney Lipid Content

The performances during the graded exercise test for HCR compared to the LCR are described in table 1. The HCR rats' initial weight was significantly lower than that of LCR rats (22%, $p < 0.05$; table 2), with the HFD not leading to significant increases in body weight above NCD levels in either group. The HCR had a significantly lower body composition ratio compared to LCR on NCD (36%, $p < 0.05$; table 2), with the HFD leading to a 2-fold increase in LCR rat body composition ratio versus NCD ($p < 0.05$; table 2) and a significant difference between LCR and HCR ($p < 0.05$; table 2). No significant differences were observed between LCR and HCR rats on NCD or following the HFD intervention in either serum triacylglycerols or free fatty acids (table 2). In addition, no differences were observed between the LCR and HCR rats or the diets in kidney tissue triacylglycerol levels (data not shown).

Table 1. Aerobic capacity

	LCR		HCR	
	NCD	HFD	NCD	HFD
Running distance, m	358 ± 22	344 ± 27	1,821 ± 73*	1,851 ± 88*
Time to exhaustion, min	23 ± 1	23 ± 1	68 ± 2*	69 ± 2*

* $p < 0.05$ HCR vs. LCR.

Intraperitoneal Glucose Tolerance Test

The HCR displayed higher insulin sensitivity as shown by decreases in both glucose and insulin area under curves (AUC) in the HCR (19 and 28%, respectively) versus LCR (each $p < 0.05$; fig. 1a and b) in response to the

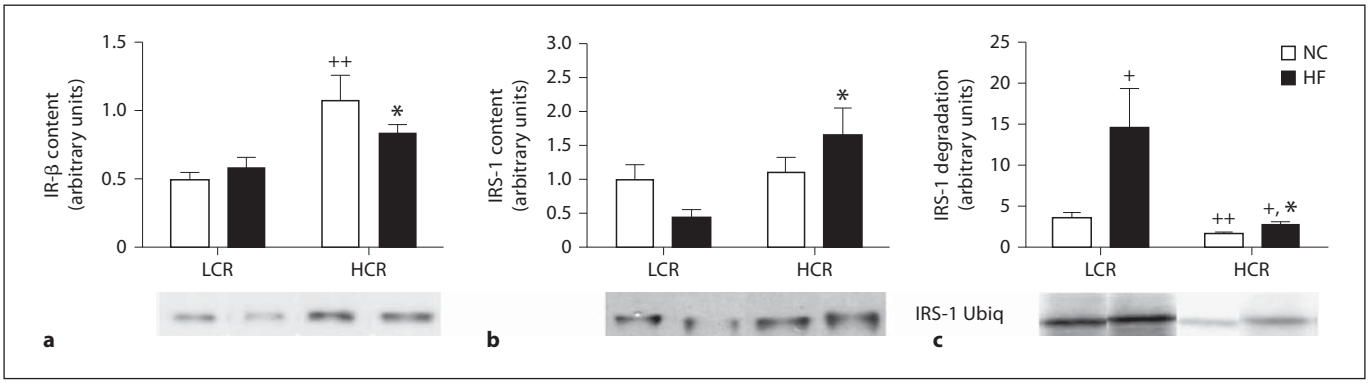

Fig. 3. Kidney tissue insulin receptor. **a** IR-β protein content. **b** IRS-1 protein content. **c** IRS-1 degradation. $^{+}$ $p < 0.05$ between NCD and HFD; $^{++}$ $p < 0.05$ between LCR and HCR on NCD; * $p < 0.05$ between LCR and HCR on HFD.

Table 2. Total body weight and serum measures

	LCR		HCR	
	NCD	HFD	NCD	HFD
Initial weight, g	279 ± 16	268 ± 10	219 ± 7**	217 ± 4**
Final weight, g	300 ± 21	295 ± 12	233 ± 10**	238 ± 8**
Weight gain, g/week	3.00 ± 0.75	3.86 ± 0.52	2.06 ± 0.6	2.93 ± 0.65
Body composition ratio	0.58 ± 0.05	1.15 ± 0.19*	0.37 ± 0.02**	0.41 ± 0.08***
Serum TAG, mg/dl	136 ± 4	135 ± 7	136 ± 8	135 ± 4
Serum FFA, mmol/l	495 ± 30	431 ± 33	533 ± 58	443 ± 51

Data collected following 7 weeks of NC or HFD.
* $p < 0.05$ LCR (HFD) vs. LCR (NCD); ** $p < 0.05$ HCR vs. LCR; *** $p < 0.05$ LCR (HFD) vs. HCR (HFD).

glucose challenge. The HFD lead to increased insulin AUC in both LCR and HCR rats, with only the 26% increase in LCR reaching significance ($p < 0.05$).

Kidney ROS Potential and Insulin Resistance

In order to control for aerobic influence on mitochondrial content, we normalized ROS potential values for citrate synthase activity as a surrogate for mitochondrial number. Under these conditions, there were no significant differences observed in kidney ROS potential between the HCR and LCR (fig. 2A). However, the HFD lead to increases of 58% ($p < 0.05$) and 69% ($p < 0.05$) in kidney ROS potential in both HCR and LCR rats, respectively. This correlated with systemic insulin AUC ($r < 0.78$, $p < 0.0004$; fig. 2b). We further looked at the contributions of NADPH oxidase activity. Of note, there were no significant differences in NADPH oxidase activity between the HC and LCR, nor was it affected by the HFD (fig. 2c).

Kidney IR-β, IRS-1 Content and IRS-1 Degradation

The HCR rats had 2-fold greater IR-β protein content compared to LCR (44%, $p < 0.002$; fig. 3a). The HFD intervention did not lead to further decreases in IR-β content in the LCR rats, and only a nonsignificant decrease in HCR rats. However, IR-β content was still significantly greater in the HCR compared to the LCR. No difference was observed in the kidney IRS-1 protein content between HCR and LCR. However, the HFD lead to a 3-fold greater IRS-1 content in the HCR compared to the LCR and a decreasing trend in the LCR relative to NCD. We further determined the degradation of kidney IRS-1 as the ratio of IRS-1 ubiquitination to total IRS-1. On NCD, the HCR rats had a 53% ($p < 0.05$) lower IRS-1 deg-

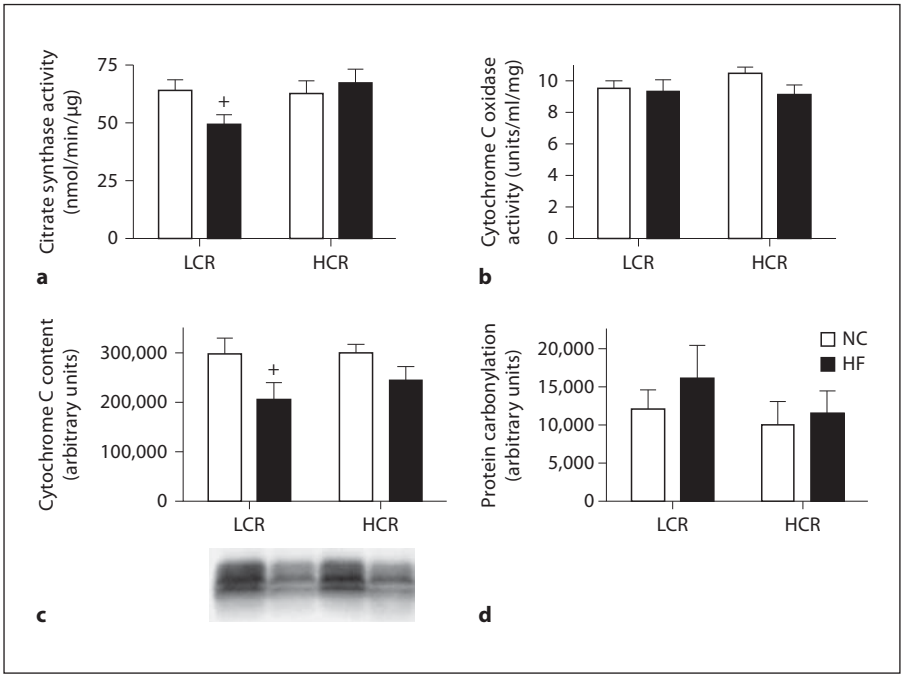

Fig. 4. Measures of mitochondrial function and oxidative stress. Citrate synthase activity (**a**) and cytochrome c oxidase activity (**b**) from kidney tissue crude mitochondrial fractions. **c** Western blot analysis of cytochrome c protein content. **d** Protein carbonylation as a marker of protein reactive aldehydes. $^{+}p < 0.05$ between NCD and HFD.

radation compared to the LCR (fig. 3b). HFD increased IRS-1 degradation in LCR rats 4-fold ($p < 0.05$), while only increasing 55% ($p < 0.05$) in HCR rats. Strikingly, a 5.5-fold difference ($p < 0.05$) in kidney IRS-1 degradation was observed between the LCR and HCR rats following the HFD intervention.

Mitochondrial Measures and Tissue Oxidative Stress

Following the 7 weeks of HFD, a 22% ($p < 0.05$) decrease in LCR rat citrate synthase activity was observed, with no additional significant findings (fig. 4a). There were no significant differences observed between the HCR and LCR rats' cytochrome c oxidase activity (fig. 4b). Additionally, cytochrome c content was similar in both strains on NCD, with a decrease on HFD only reaching significance in LCR rats (31%, $p < 0.05$; fig. 4c). Finally, no significant differences were observed in kidney tissue oxidative stress as measured by protein carbonylation (fig. 4d).

Discussion

This investigation examined the impact of intrinsic aerobic capacity on HFD-induced generation of renal ROS and associated reductions in renal IRS-1 levels and systemic insulin metabolic sensitivity. In this context, ROS production, in relation to IRS-1 levels/degradation, was assessed in the HCR and LCR rat model fed a HFD. These 2 phenotypes of disparate intrinsic aerobic capacity manifest other dynamic differences in the physiology of several insulin-sensitive tissues [11, 20, 21, 29], while also exhibiting differential effects in the capacity to withstand dietary and other metabolic challenges [11]. Intrinsic aerobic capacity is known to be a determinant of insulin sensitivity [10, 11], and insulin resistance is associated with the development and progression of CKD [1–4]. Although this is largely a descriptive study, the data from this investigation indicate that increased aerobic capacity can mitigate a HFD-induced increase in renal ROS-associated proteosomal degradation of IRS-1 docking protein, whose cellular content is crucial for maintenance of normal insulin metabolic signaling. Further, increased aerobic capacity is protective against subsequent decreases in systemic insulin sensitivity following metabolic challenge. These findings in the kidney are consistent with systemic and skeletal muscle insulin signaling where increased aerobic capacity is protective against changes in oxidative stress and insulin signaling [11, 30, 31].

Current evidence suggests that the role of insulin action in the kidney is primarily focused on sodium reabsorption, with additional roles in glucose metabolism, NO production and maintenance in glomerular struc-

ture and function [32]. Recent interest has grown in insulin signaling and action in the kidney as a contributor to systemic insulin resistance. Thus, the observed increase in IRS-1 protein degradation and decreased IR-β content in the kidney of the insulin-resistant LCR rat model is novel. Although our data cannot distinguish between the section of the nephron, the divergent IRS-1 protein content between the aerobic phenotypes and the dramatic augmentation of IRS-1 degradation in the LCR rat following the metabolic challenge (that is, HFD) are of particular interest. Collectively, our data support that the higher intrinsic aerobic capacity of the HCR rat is protective from changes in kidney insulin receptor substrate degradation in basal and HFD conditions. This data supports and extends previous observations that a higher intrinsic aerobic capacity offers protection from the development of systemic insulin resistance [11, 21].

Resistance to the metabolic actions of insulin can be mediated through a degradation of IRS-1 via redox-sensitive serine phosphorylation of IRS-1 and decreased downstream signal transduction [17, 33]. We observed an increase in kidney ROS potential following the HFD from both phenotypes in this study. Elevated ROS has been shown to activate many of the same signaling cascades, including the stimulation of serine kinases which can lead to alterations in the IRS-1 phosphorylation and subsequent proteosome degradation [34, 35]. The strong correlation between ROS potential and insulin AUC suggests a potential relationship between hyperinsulinemia and the oxidative stress state within the kidney as a putative mechanism for increased IRS-1 degradation.

Tissue ROS production is primarily driven by either mitochondrial uncoupling and/or the NADPH oxidase enzyme complex. Kidney mitochondrial ROS production has been described in models of high lipid exposure and hyperglycemia [15, 16, 36]. This mitochondrial ROS production is frequently described as a consequence of altered or inappropriate mitochondrial handling and metabolism of lipids, often with significant lipid accumulation, accompanied by subsequent uncoupling of the respiratory chain. Recent data suggest altered renal mitochondrial function and significant renal lipid accumulation, both in rodent models of diabetes and after a HFD, are causal for development and progression of CKD [37–39]. Herein, we report that no significant lipid accumulation is observed following 7 weeks of HFD intervention in the LCR. However, the significant decreases in citrate synthase and cytochrome c content following HFD support initiation of mitochondrial dysfunction within the kidney. Additionally, the lack of significant protein carbonylation, as a marker of tissue oxidative stress, may suggest that our data are early in the course of high fat-induced renal ROS levels.

The increase in ROS could come from several sources. The NAD(P)H oxidase enzyme is capable of significant ROS production. Our laboratory and others have described activation of the NAD(P)H oxidase family and its involvement in various facets of CKD and diabetic kidney disease [22–24, 26, 40]. However, in the LCR rats it appears that the mitochondria are the primary source of ROS production, as we saw no difference in NAD(P)H oxidase activity. Collectively, these data suggest that aerobic capacity is important for the maintenance of normal renal mitochondrial ROS generation.

In summary, although this is a largely descriptive study, our data suggest that increased intrinsic aerobic capacity is protective against HFD-induced decreases in insulin receptor substrate through maintenance of appropriate mitochondrial function. Both decreased aerobic capacity and HFD potentially contributed to increased renal ROS potential, with ROS as a significant axis in the alteration of IRS-1 proteosomal degradation (ubiquitination).

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